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ISOFORMS OF TURKEY PROLACTIN: EVIDENCE FOR DIFFERENCES IN GLYCOSYLATION AND IN TRYPTIC PEPTIDE MAPPING*

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Abstract—1. Three isoforms of turkey pituitary prolactin have been isolated, including a nonglycosylated isoform of 22,500 mol. wt and two glycosylated isoforms of 24,500 mol. wt.

2. The glycosylated turkey prolactins differed in carbohydrate composition, with one isoform apparently containing only *O*-linked carbohydrate.

3. Tryptic peptide maps showed a few peptides distinctly different among the three prolactin isoforms.

4. Amino acid sequencing of the first 40 residues of the three prolactin isoforms showed arginine at position 24 and histidine at position 27, for the nonglycosylated form, but no identifiable amino acids were detected at this position for the glycosylated isoforms.

INTRODUCTION

Prolactin (PRL) is a polypeptide belonging to the family of hormones including growth hormones and placental lactogens which share certain biological, immunological and structural features. Prolactin, the product of a single gene in humans, rats and turkeys, is involved in a wide array of metabolic events such as the regulation of growth and development, the regulation of reproductive functions, the production of 1,25-dihydroxycholecalciferol, the control of water and electrolyte balance and the growth of lymphoid tissue. The basis for the diversity of these PRL functions has not been explained in terms of its molecular structure. Although charged forms (Oetting and Walker, 1985; Oetting *et al.*, 1986) and cleaved forms (Oetting and Walker, 1985; Clapp *et al.*, 1989) of PRL have been described, they have not been associated with discrete biological functions. The clearly demonstrated influence on physiological events which PRL exercises in avian species makes the turkey an excellent experimental model for the investigation of the mechanisms of PRL control. The interaction of light and dark cycles with PRL secretion and the hormone's influences on egg laying and incubation behavior are well known phenomena in birds, but have not been defined on a biochemical basis. Prolactin control in mammalian systems suffers the disadvantages of greater complexity and more subtle changes in PRL secretion in response to stimuli.

We have previously reported the occurrence of multiple species of the turkey PRL molecule in pituitary extracts separated by an improved purification procedure (Proudman and Corcoran, 1981). Since these forms differed by 2 k in mol. wt on SDS–PAGE, they could not be desamido or simple charge difference forms. The results of amino acid analysis ruled out significant differences in the polypeptide lengths. Following a report (Lewis *et al.*, 1984) describing the presence of asparagine-linked glycosylation on the ovine PRL molecule, we investigated the possibility that turkey PRL may occur in a glycosylated form. We report here the identification of three monomeric isoforms of turkey PRL, two of which are glycosylated. The two glycosylated isoforms apparently result from different kinds of glycosylation rather than from a difference in the amount or accessibility of the carbohydrate. A preliminary report of these findings has been presented (Corcoran and Proudman, 1987).

*The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

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Abbreviations used—CHO, carbohydrate; con-A, concanavalin A; G–PRL(R), glycosylated turkey prolactin bound by con-A; G–PRL(U), glycosylated turkey prolactin not bound by con-A; HPLC, high-performance liquid chromatography; LH, luteinizing hormone; NG–PRL, non-glycosylated turkey prolactin; PITC, phenylisothiocyanate; PRL, prolactin; SDS–PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TEA, triethylamine; TCA, trichloroacetic acid.

MATERIALS AND METHODS

Tissue source

Pituitaries were collected at a packing house where young, sexually-immature market turkeys (*Meleagris gallopavo*) and mature breeders of both sexes were included. Tissues were frozen at collection and stored at −70°C until utilized.

Purification procedure

The PRL isohormones were purified using a modification of our previous procedure (Proudman and Corcoran, 1981). The source of pituitaries was an heterogeneous population

of turkeys including both sexes and mature and immature birds. This cross-section of the sex and age should yield the maximum number of different PRL isoforms. Pituitaries were briefly extracted twice at pH 5.5 in 0.1 M sodium acetate buffer containing 0.05 M lactose and the following inhibitors: 10 mM 4-aminobenzamidine dihydrochloride (protease inhibitor); 2.5 mM mercuric chloride and 0.1 mM 4-chloromercuribenzoic acid (glycosidase inhibitors); and 2 mg/l phosphotungstic acid (neuraminidase inhibitor). The homogenate residue, containing the PRL, was then extracted for 2 hr at 4°C with 0.1 M $NaHCO_3$, pH 9, containing lactose, 4-aminobenzamidine dihydrochloride and phosphotungstic acid, as above. The supernatant fraction was concentrated and chromatographed on a 2.6 × 95 cm column of Sephacryl S-200 equilibrated and eluted with 0.1 M NH_4HCO_3–0.05 M NaCl, pH 9, containing lactose and phosphotungstic acid as above. The PRL fractions were purified by preparative isotachophoresis as described previously (Proudman and Corcoran, 1981). The "prolactin II" fraction (Proudman and Corcoran, 1981) was dialyzed by ultrafiltration and applied to a 1.6 × 4.5 cm column of con-A Sepharose and eluted with the dialysis buffer (0.02 M Tris–HCl with 0.5 M NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$, pH 7.4). The retarded PRL was eluted with the starting buffer plus 0.05 M α-methyl-D-glucoside. Treating the column with 0.5 M α-methyl-D-glucoside did not result in further protein elution. The PRL fractions unretarded and retarded on con-A were then separately chromatographed on a 2.6 × 200 cm column of Sephacryl S-200, as described above. The PRL isohormones were extensively dialyzed by ultrafiltration against 1 mM NH_4HCO_3, pH 9, and lyophilized.

Amino acid sequencing

Samples of the three PRL isoforms, approximately 500 μg each, were sequenced by a Beckman 890C sequencer using the 0.1 M Quadrol protein program (Beckman) with the addition of 1.5 mg of polybrene. The PTH amino acids resulting from the sequencing procedure were separated by HPLC on a DuPont 0.46 × 25 cm Zorbax C-18 column at 58°C, using a linear gradient from 20 to 42% acetonitrile (Zimmerman *et al.*, 1977) in 0.02 M sodium acetate, pH 5.5. Two sequence analyses were performed on NG–PRL and G–PRL(R) and one on G–PRL(U). The first 10 residues of each isoform were also sequenced by an independent laboratory using a gas phase sequencer and detection on a microbore cyano HPLC column.

Carbohydrate determination

N- and *O*-linked carbohydrates were determined as follows: neutral sugars of the *N*-linked CHO were identified following hydrazinolysis (Takasaki *et al.*, 1982) of 200 μg samples. Following the conversion and reduction in the hydrazinolysis procedure, the samples were dried and hydrolyzed in 200 μl of 2 N HCl for 5 hr at 100°C in an evacuated tube. The sample was dried and analyzed using the dansyl hydrazine detection method described below. *O*-Linked CHO was prepared from 200 μg samples utilizing the 4 M HCl/4 M TFA hydrolysis procedure described by Takemoto *et al.* (1985), followed by drying and identification of the neutral sugar composition by a modification of the dansyl hydrazine method of Mopper and Johnson (1983). The dansyl hydrazine method was modified as follows: 100 μl of water, 10 μl of 10% (w/v) TCA and 50 μl of a 5% (w/v) solution of dansyl hydrazine in acetonitrile was added to the dry sample, the tube was sealed and heated at 65°C for 20 min. The sample was diluted with 1.8 ml of water and applied to a Sep-Pak C-18 column (Waters) which had been activated by rinsing with 2 ml of 40% acetonitrile followed by 1 ml of water. Two ml of 10% (v/v) acetonitrile was applied to the column and the sample was then eluted with 2 ml of 40% acetonitrile. The eluent was dried, redissolved in 200 μl of 22% acetonitrile, and separated by HPLC using a Waters uBondapak C-18 column (0.46 × 15 cm). The chromatography conditions were: solvent A, 0.02 M acetic acid; solvent B, acetonitrile; flow rate, 1.2 ml/min; gradient, equilibrated with 16% B and eluted for 2 min, followed by a linear gradient to 19% B in 16 min, 19% to 60% B in 2 min, 60% to 80% B in 5 min, 80% B for 10 min, 80% to 100% B in 5 min, 100% B for 6 min, 100% to 16% B in 3 min, followed by 15 min re-equilibration before the next injection. Identification of sugars in unknowns was based on elution times of standards run on the same day. A Kratos Model FS970 fluorescence detector (excitation, 360 nm; emission, >470 nm) was used.

Amino sugar determination

Hydrolysis of samples (150 μg) was performed as for *O*-linked carbohydrates, above. The following protocol for amino sugar determination is a modification of a procedure provided by Waters Chromatography Division, Milford, MA. The dried samples were dissolved in 200 μl of ethanol:water:TEA (2:2:1 v/v/v), re-dried, 60 μl of a solution of ethanol, water, TEA, and phenylisothiocyanate (7:1:1:1 v/v/v/v) was added, and the sample was incubated at room temperature for 20 min in the dark to produce the phenylthiocarbamyl derivatives of the amino sugars and amino acids. After re-drying, the sample was dissolved in 50 μl of 5% acetonitrile and injected onto a 0.46 × 15 cm C-18 Resolve column (Waters) and chromatographed using the following protocol: solvent A, 0.14 M sodium acetate containing TEA (700 μl/l), pH 5.3; solvent B, 60% acetonitrile; flow rate, 1 ml/min at 10% B; temperature, 38°C. After injection, B increased to 51% in 10 min following an exponential concave gradient (curve #5 on the Waters Model 830 Data Module), then B increased linearly to 100% in 0.4 min. After 1.3 min, the flow rate increased to 1.5 ml/min and B declined to 10% over 0.5 min and remained at that level for 7.5 min. The column was re-equilibrated at a flow rate of 1 ml/min, 10% B. Detection was at 254 nm.

Sialic acid determination

Sialic acid was released from 5 to 10 μg of each PRL isoform by mild acid hydrolysis in 25 mM sulfuric acid for 1 hr at 60°C. The samples were applied to an Aminex A-28 anion exchange column prepared and eluted as described by Shukla and Schauer (1982). Sialic acid was quantified relative to the peak area of sialic acid standards.

Tryptic peptide mapping

Approximately 350 μg samples of lyophilized PRLs were denatured at room temperature overnight in 20 μl of 8 M urea dissolved in 0.46 M Tris–HCl, pH 8, containing 1.15 mM $CaCl_2$. The samples were then diluted 1:10 with water and 3.5 μg of TPCK trypsin were added and the samples allowed to stand at room temperature for about 6 hr. A second aliquot of 3.5 μg of trypsin was added and the samples were incubated overnight at room temperature, then applied to a 0.46 × 15 cm uBondapak phenyl column (Waters). Chromatography conditions were: solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile; flow rate, 0.7 ml/min at 10% B; gradient, 10% B for 2 min following injection, followed by a linear gradient to 27% B in 30 min, 27–41% B in 18 min, 41–90% B in 5 min, 5 min at 90% B, 90–10% B in 4 min, followed by re-equilibration. Chromatography was at room temperature and detection was at 206 nm with 1.0 AU full scale. Peaks were collected and those which differed among the PRL isoforms were analyzed for CHO content as described above for *O*-linked CHO.

Prolactin radioreceptor assay

The microsomal fraction of a chicken kidney homogenate was prepared as described by Shiu *et al.* (1973) and displace-

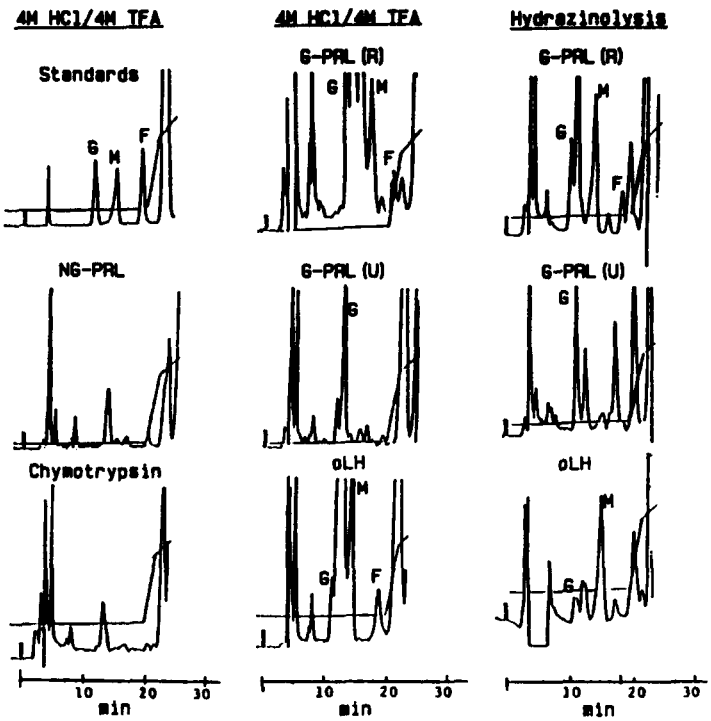

Fig. 1. Dansylhydrazine derivatives of the neutral sugars were produced as described under Methods using either 4 M HCl–4 M TFA hydrolysis to cleave the polypeptide chain or the hydrazinolysis procedure described. Chymotrypsin (no carbohydrate content) and oLH (*N*-linked carbohydrate) are included for comparison. Fluorescence excitation was at 360 nm and emission above 470 nm.

ment of radioiodinated ovine PRL by turkey PRL isohormones was measured by radioreceptor assay (Leung *et al.*, 1984).

RESULTS

Three isoforms of turkey PRL were purified by the procedure described above, in addition to the "prolactin I" fraction described in our earlier study (Proudman and Corcoran, 1981) and presumed to be deamidated PRL. Our current procedure eliminated the "large" PRL aggregates previously formed during purification. A typical yield from 25 g of pituitaries was 8.2 mg of "prolactin I" and 9.5 mg of "prolactin II". Further purification of "prolactin II" yielded the three isohormones. One glycosylated isoform [G–PRL(R)] retarded on con-A was eluted with 0.05 M glucopyranoside and had an apparent mol. wt on SDS–PAGE of 24.5 k. Two other isoforms were unretarded by con-A and were separated by gel filtration. One (NG–PRL) was not glycosylated and had an apparent mol. wt of 22.5 k, while the other [G–PRL(U)] was glycosylated and had an apparent mol. wt of 24.5 k. Yields of these isohormones were: 2.35 mg NG–PRL, 1.65 mg G–PRL(U), and 0.98 mg G–PRL(R). An additional 1.85 mg of mixed NG–PRL and G–PRL(U) was incompletely resolved by gel filtration.

Isohormone carbohydrate composition

HPLC chromatograms of approximately equal amounts of the dansylated hydrazine derivatives of the neutral sugars are shown in Fig. 1. Hydrazinolysis of G–PRL(R) and G–PRL(U) showed that the G–PRL(U) form contained more galactose than a similar amount of the G–PRL(R) form. No mannose or fucose were detected in the G–PRL(U) form, but these sugars were clearly present in the G–PRL(R) form. Several peaks representing partially degraded material were present in both cases. The NG–PRL form lacked CHO. Chymotrypsin, a protein lacking glycosylation and analyzed as a control, showed no peaks corresponding to the neutral sugars. Analysis of ovine LH, known to contain *N*-linked CHO, showed the predominant sugar to be mannose, consistent with its known CHO content. Fucose, destroyed in LH by the hydrazinolysis procedure, was present after the milder TFA–HCl hydrolysis. Fucose was found in G–PRL(R) following hydrazinolysis, suggesting that the CHO in the PRL may be more protected than the CHO is in LH. The TFA–HCl cleavage of CHO in the G–PRL(U) found galactose to be the predominant sugar. No mannose was observed. Galactose, mannose and fucose were all observed on G–PRL(R), similar to ovine LH. Table 1 summarizes the occurrence and relative amounts of carbohydrates present in the three PRL isoforms. Figure 2 presents chromatograms of the HPLC amino sugar determination for G–PRL(R) showing glucosamine and galactosamine, for G–PRL(U) showing only glucosamine. Bovine LH, known to contain *N*-linked carbohydrate (Sairam, 1983), is included here as a reference. No amino sugar was found on the NG–PRL (chromatogram not shown). The acid hydrolysis required for amino sugar release

Table 1. Relative carbohydrate compositions of three turkey prolactin isoforms

Isoform	Carbohydrate					
	Man	Gal	Fuc	$GlcNH_2$	$GalNH_2$	NANA
G–PRL (R)	++	+	+	++	+	+
G–PRL (U)	–	+++	–	+++	–	+
NG–PRL	–	–	–	–	–	–

Man = mannose, Gal = galactose, Fuc = fucose, $GlcNH_2$ = glucosamine, $GalNH_2$ = galactosamine, NANA = sialic acid. + = relative amount of CHO found; – = not found.

removes acetylation from the amino sugar group and also breaks many peptide bonds freeing amino acids which represent most of the peaks in the chromatograms. Sialic acid was detected in both glycosylated turkey PRL isoforms. The G–PRL(R) had 8.7 ± 0.5 ng sialic acid per μg protein (0.9%), while the G–PRL(U) had 11.4 ± 0.2 ng/μg (1.1%). No sialic acid was detected on the NG–PRL isoform.

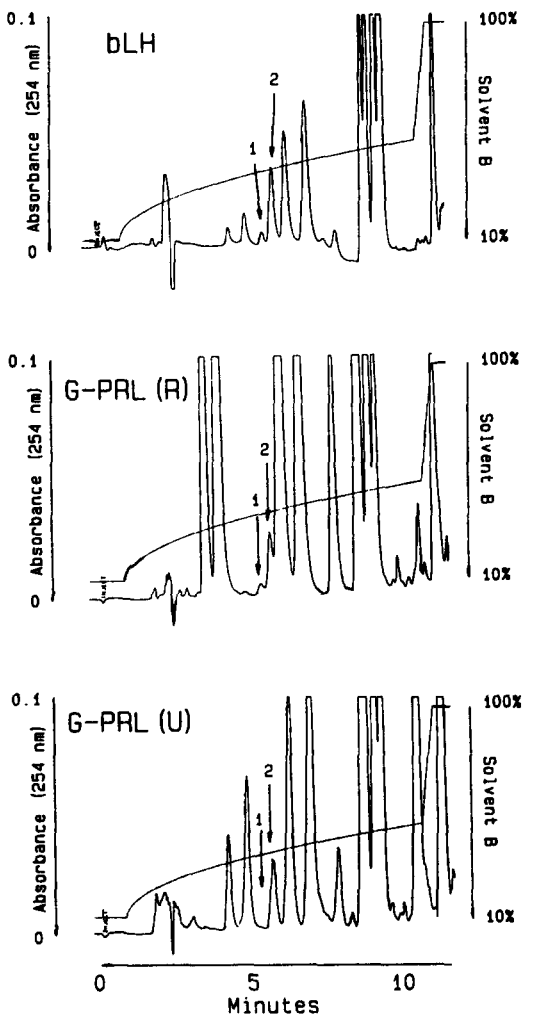

Fig. 2. Amino sugar determination. Following acid hydrolysis, HPLC was performed on the PTC derivatives of the released sugars and amino acids (see Methods). Positions of the amino sugars are indicated by arrows: (1) is galactosamine; (2) is glucosamine. Bovine luteinizing hormone (bLH), which contains *N*-linked carbohydrate, is shown for comparison.

Primary sequence determination

The *N*-terminal amino acid sequence of NG–PRL is compared with the sequence of chicken PRL and several mammalian PRLs (Table 2). The three turkey PRL isoforms shared all but two of the first 40 amino acid residues. Exceptions were Arg-24 and His-27 which were clearly present in the NG–PRL isoform, but were not detected in the two glycosylated forms. Failure to detect any residue at these sites in the glycosylated forms may indicate that these residues which may not be Arg or His are covalently bound to either CHO, and thus not retained in the sequencer, or to a residue elsewhere in the polypeptide chain. Comparison of the turkey PRL sequence with those of chicken (Hanks *et al.*, 1989), human (Cooke *et al.*, 1981), ovine (Li *et al.*, 1970), bovine (Sasavage *et al.*, 1982), rat (Seeburg *et al.*, 1977), and porcine (Li, 1976) PRLs reveals that the closest homology in the first 40 residues is to chicken (90%) and porcine (85.5%) PRLs.

Tryptic peptides

Tryptic peptide maps of the three PRL isoforms are shown in Fig. 3. Peptide C was observed only in the G–PRL(U) isoform, while peptides 11, 13 and 14 were found primarily in the NG–PRL. Peptides 17 and 18 were shared by G–PRL(R) and NG–PRL, but were not found in the G–PRL(U), while peptides A and B were shared by the two glycosylated isoforms, but not by the non-glycosylated form. Finally, peptide 21 was found in G–PRL(U) and NG–PRL, but not in G–PRL(R). Two residues of the first forty, Arg-24 and His-27, could be identified from the non-glycosylated form but not in either of the glycosylated forms. Arg-24 is trypsin sensitive and would result in two new peptides not seen in the glycosylated forms. One of these would be a tripeptide, represented perhaps by peptide 13 or 14 on the NG–PRL chromatogram in Fig. 3.

Determination of neutral sugars on several of the peptides found galactose on peptide C of the G–PRL(U) and mannose and galactose on peptide 9 and mannose alone on peptide 15 of the G–PRL(R) isoform. No CHO was found on peptides examined from the NG–PRL.

Prolactin radioreceptor activity

All three isoforms were more potent in a PRL radioreceptor assay than our earlier preparation (Proudman and Corcoran, 1981), which presumably consisted of a mixture of these isoforms (Fig. 4). The NG–PRL had the greatest potency in the radioreceptor assay, with an ED_{50} of 4.9 ng. By comparison, ovine PRL standard had an ED_{50} of 5.2 ng in this assay.

Table 2. Comparison of the primary amino acid sequence for the first 40 residues of turkey prolactin with those of chicken and mammalian prolactins

Species	1					6					11					16				
Turkey	L	P	I	C	S	S	G	S	V	N	(C)	Q	V	S	L	G	E	L	F	D
Chicken	L	P	I	C	P	I	G	S	V	N	C	Q	V	S	L	G	E	L	F	D
Porcine	L	P	I	C	P	S	G	A	V	N	C	Q	V	S	L	R	D	L	F	D
Human	L	P	I	C	P	G	G	A	A	R	C	N	V	T	L	R	D	L	F	D
Bovine	T	L	V	C	P	N	G	P	G	N	C	Q	V	S	L	R	D	L	F	D
Ovine	T	P	V	C	P	N	G	P	G	D	C	Q	V	S	L	R	D	L	F	D
Rat	L	P	V	C	S	G	G	·	·	D	C	Q	T	P	L	P	E	L	F	D

Species	21					26					31					36				
Turkey	R	A	V	R*	L	(S)	H*	Y	I	H	F	L	(S)	(S)	E	I	F	N	E	F
Chicken	R	A	V	K	L	S	H	Y	I	H	Y	L	S	S	E	I	F	N	E	F
Porcine	R	A	V	I	L	S	H	Y	I	H	N	L	S	S	E	M	F	N	E	F
Human	R	A	V	V	L	S	H	Y	I	H	N	L	S	S	E	M	S	F	E	F
Bovine	R	A	V	M	V	S	H	Y	I	H	D	L	S	S	E	M	F	N	E	F
Ovine	R	A	V	M	V	S	H	Y	I	H	N	L	S	S	E	M	F	N	E	F
Rat	R	V	V	M	L	S	H	Y	I	H	T	L	Y	T	D	M	F	I	E	F

Amino acid code: A = Ala, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, Y = Tyr; · = not present, ( ) = tentative determination, E or N could be Q or D, respectively. *Denotes R and H residues found in the nonglycosylated isoform only; no residue could be determined for the two glycosylated isoforms.

DISCUSSION

The data presented here show that monomeric turkey PRL is present in the pituitary in three isoforms which differ in mol. wt, CHO composition and possibly peptide content. Great care was taken to preserve the PRL forms in their native states, therefore no reducing agents or cysteine modifying agents were employed. For CHO analysis the 4 M HCl/4 M TFA cleavage yielded cleaner chromatograms than hydrazinolysis due to reduced cleavage of polypeptide bonds. This work presents direct evidence for two distinct glycosylated isoforms of PRL, one of which appears to contain only *O*-linked carbohydrate, and the first evidence of sialic acid on a glycosylated PRL. Our conclusion that G–PRL(U) contains only *O*-linked glycosylation is based on the absence of mannose and fucose. The content of mannose, galactose, fucose, glucosamine and galactosamine in the G–PRL(R) isoform is consistent with the known composition of *N*-linked carbohydrates such as those found on pituitary gonadotropic hormones (Sairam, 1983). Our results show galactose, but not mannose, to be present in a tryptic peptide fraction that is only found in digests of the G–PRL(U) isoform (peptide C, Fig. 3). However, our peptide mapping data do not necessarily demonstrate unique peptide sequences for the turkey PRL isoforms since it is possible that the presence of carbohydrates may modify the accessibility of arginine or lysine residues to trypsin. Indeed, we were unable to achieve consistent peptide maps without stringent denaturation of the glycosylated PRLs in urea. A recent report (Lewis *et al.*, 1989) has demonstrated the existence of two forms of glycosylated human PRL which also differ in binding to con-A and exhibit a difference in the elution time of a single tryptic peptide during peptide mapping, but the carbohydrates present on these isoforms have not been characterized.

Our sequence data show turkey PRL to differ from chicken PRL in only three of the 40 *N*-terminal amino acids, with two of these being conservative substitutions. Like chicken, bovine and rat PRLs, turkey PRL does not contain an asparagine residue at position 31, the designated site of *N*-linked glycosylation in the porcine, ovine and human hormones. We suggest that two possible sites for *N*-linked glycosylation may exist in PRL at Asn-X-Cys sites where Cys may perform a function similar to Ser/Thr in the *N*-linked glycosylation procedure (Foster and Davie, 1984). Since these cysteines are normally involved in disulfide bond formation, such glycosylation would interfere with the disulfide cross-linking if it preceded the disulfide bond formation. Loss of a single cross-link would result in significant conformational changes in the PRL molecule which may be directly responsible for altered binding to receptors and therefore altered activities and stabilities. Our results also suggest that *O*-linked glycosylation may occur in the PRL of some species. This *O*-linked glycosylation may be easily lost during purification through exposure to high pH or to lysosomal hydrolytic enzyme activity at moderately low pH. Addition of glycosidase and neuraminidase inhibitors and avoidance of alcoholic extraction and protein precipi-

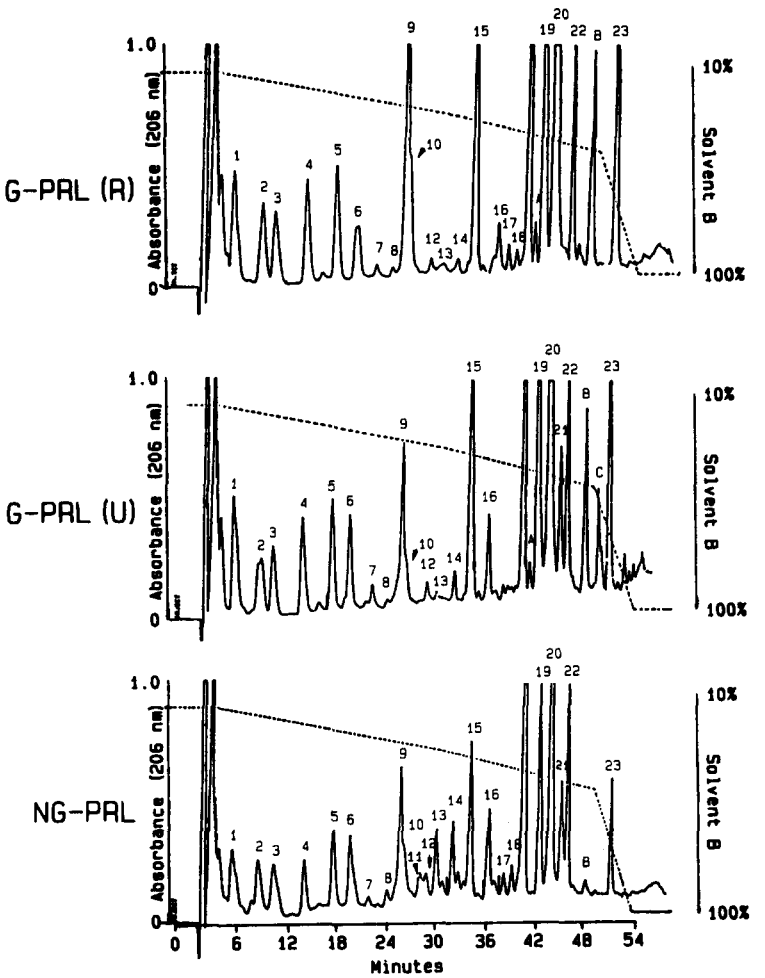

Fig. 3. Tryptic peptides of turkey PRL isoforms eluted as described in Methods.

tation steps may have enhanced our recovery of this PRL isoform from the turkey pituitary.

Our evidence that glycosylated turkey PRLs exhibit lower receptor binding activity than the nonglycosylated isoform is consistent with reports that glycosylation decreases the biological activity of PRL. For example, glycosylated ovine PRL was shown to have 20, 24, or 80% of the activity of the nonglycosylated form in rabbit lactogen receptor binding, Nb_2 lymphoma cell, and mouse mammary gland explant assays, respectively (Markoff *et al.*, 1988).

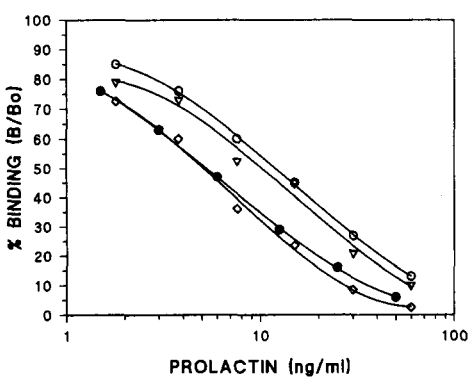

Fig. 4. Radioreceptor assay potency of turkey PRL isoforms compared to an ovine PRL standard. (●) Ovine PRL standard $ED_{50} = 5.2$ ng; (◇) NG–PRL, $ED_{50} = 4.9$ ng; (△) G–PRL(R), $ED_{50} = 8.7$ ng; (○) G–PRL(U), $ED_{50} = 10.8$ ng.

The role of individual PRL isoforms in promoting the diverse physiological actions of PRL is unknown, as is the manner in which PRL isoforms arise from a single gene and how their secretion from the pituitary is controlled. Our data show that in excess of 50% of the monomeric PRL isolated from the turkey pituitary is glycosylated (see Results above). Glycosylated PRL constitutes 30–40% of the PRL present in porcine pituitary (Pankov and Butnev, 1986) and a lesser proportion of human (Lewis *et al.*, 1985) and ovine (Lewis *et al.*, 1984) pituitary PRL. Glycosylated PRL is found in the circulation of humans (Markoff and Lee, 1987; Sinha *et al.*, 1984) and pigs (Sinha *et al.*, 1988). Circulating levels of glycosylated PRL have been shown to vary with age in the pig, independent of changes in the nonglycosylated form (Sinha *et al.*,

1988). Higher ratios of glycosylated to nonglycosylated PRL were found during the period from birth to two months of age, with ratios decreasing thereafter.

The fate of prolactin after binding to its receptor has not been elucidated. Recent work (Holt and Hart, 1986; Hart *et al.*, 1988; Jackson and Tjian, 1989) provides evidence for a nuclear or perinuclear location of proteins bearing *N*-acetylglucosamine linkages in *O*-linked glycosylations on several proteins. A direct or indirect role of these proteins in activation of gene transcription is a possibility. Prolactin, like many hormones, has growth-promoting activities (e.g. mammary tissue proliferation, lymphoid tissue growth). In addition, PRL is involved in metabolic processes (e.g. electrolyte water balance, 1,25-dihydroxycholecalciferol production). Insulin, an intensely studied hormone, also shows both growth-promoting and metabolic activities. Assuming that cells frequently use common mechanisms for parallel kinds of operations, some new information on insulin's activity inside the cell may provide some insight into the action of PRL. The insulin molecule itself has been shown to enter the nuclei of certain target tissue cells (Solar *et al.*, 1989) where it may be associated with the enhanced production of selective messenger RNA molecules. Similarly, prolactin's growth-promoting activities may also be the result of nuclear effects of an isoform such as the putative *O*-glycosylated turkey PRL reported here. These and other observations suggest important biological roles for glycosylated and nonglycosylated PRL isoforms.

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